Effect of Niceritrol on Streptozocin-Induced Diabetic Neuropathy in Rats

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Niceritrol, a drug with peripheral tissue vasodilatory and serum lipid-lowering activity, was administered for 2 mo to rats with streptozocin-induced diabetes. Physiological and biochemical studies were subsequently conducted on rat nerve tissue. A markedly lower value of ~47% in sciatic nerve blood flow (SNBF) was detected in an untreated diabetic (DC) group than in a nondiabetic control group (CC). A significant delay in caudal motor nerve conduction velocity (MNCV) and significantly higher glucose, sorbitol, and fructose values were observed in the sciatic nerve and serum lipids. In contrast, a niceritrol-treated diabetic (DN) group had significantly higher SNBF, MNCV, and sciatic nerve *myo*-inositol values and lower serum triglyceride levels than group DC. No differences between these two groups were noted in glucose, sorbitol, and fructose levels in the sciatic nerve, or in cholesterol and glucose in serum. These findings suggest that niceritrol has a clear inhibitory effect on the development of delayed MNCV in the diabetic rat, which may be due to reduced nerve blood flow and/or decreased nerve *myo*-inositol levels. *Diabetes* 41:587–91, 1992

The causes of neuropathy, a complication of diabetes, are thought to be metabolic factors that mainly involve abnormal metabolism of sorbitol and fructose (1,2), both intracellular reduction metabolites of glucose and a decrease in the *myo*-inositol content in nerves (3), or vascular factors derived from microangiopathy in peripheral nerves (4). However, the exact mechanism of onset of neuropathy has not yet been clarified.

According to recent reports, abnormalities such as decreased energy use in peripheral nerve tissue (3), a rise in the sodium level of axons (5), and reduced axon transport velocity (6,7) occur in in vivo neuropathy secondary to experimental diabetes. These values have become important clues in establishing the etiology of diabetic neuropathy.

Nicotinic acid, a member of the vitamin B group, has long been recognized as a drug with serum lipid-lowering (8) and peripheral vasodilatory activity (9). According to recent reports, nicotinic acid inhibits platelet aggregation and may affect the prostaglandin metabolism system that includes accelerated prostaglandin I_2 (PGI_2) production (10), inhibition of thromboxane A_2 (TXA_2) formation (11), and accelerated prostaglandin D_2 (PGD_2) production (12). In our study, niceritrol (13), an ester derivative of nicotinic acid, was administered to rats with streptozocin-induced diabetes, and physiological and biochemical investigation were conducted to determine its effect on the onset and progress of diabetic neuropathy.

RESEARCH DESIGN AND METHODS

Six-week-old male KBL Wistar rats (SPF, Kitayama Lab, Kyoto, Japan), mean body weight 247.5 ± 1.1 g (mean ± SE), were used for the experiments after they were reared for >1 wk in sets of five connecting stainless steel cages, 1 animal/cage. The rats were kept in an aseptic animal room at a room temperature of 20–24°C and a humidity of 40–70%, with a 12-h lighting cycle and 12 fresh air changes/h. The laboratory chow was solid food (CRF-1, Oriental Yeast, Tokyo, Japan), and tap water was freely available. Diabetes was induced by intraperitoneal injection of streptozocin (75 mg/kg body wt). The drug was dissolved in citric acid buffer (pH 7.4, 15 mg/ml) immediately before injection.

After 2 wk of streptozocin administration, diabetic rats (serum glucose >11.1 mM [199.8 mg/dl]) were selected

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at random and divided into two groups. A control group (group DC) had free access to laboratory chow and water without treatment for 8 wk. The remaining rats also had free access to laboratory chow and water and were maintained with oral administration of niceritrol at a dose of 300 mg/kg (10 ml · kg^{-1} · day^{-1}) by gavage for 60 days (group DN). One group of normal rats was treated with niceritrol (group CN) in the same manner as the diabetic rats and had free access to laboratory chow and water, and a control group (group CC) was untreated and had free access to laboratory chow and water.

Motor nerve conduction velocity (MNCV) was measured in the most rapidly conducting fibers of the rat-tail nerve supplying the segmental muscle by the method of Miyoshi and Goto (14), as described in another study (2). The rats were kept on a heated pad in a room maintained at 25°C to maintain a constant rectal temperature of 37°C. After the intraperitoneal injection of sodium pentobarbital (30–40 mg/kg body wt), MNCV was determined with a Neuropak NEM-3102 instrument (Nihon-Koden, Osaka, Japan) 8 wk after the initiation of treatment. The tail of the rat was kept in a 37°C liquid paraffin bath controlled by a thermostat to maintain a constant subcutaneous temperature of the rat tail, which was measured with a thermometer (Nihon-Koden). As a standard measurement procedure, the rat-tail nerve was stimulated at two points. The first stimulus point was 1 cm from the anus, and the second stimulating electrode was inserted 5 cm from the first stimulus place. Then the coaxial needle electrode was inserted into the segmental muscle of the tail, 4 cm from the second stimulus point.

The muscle action potential induced by the two-point stimulation of the longitudinal nerve trunk of the rat tail was recorded. Then, the conduction velocity was calculated by dividing the tail-nerve distance between the two stimulus points by the latency difference.

Measurement of sciatic nerve blood flow (SNBF) was conducted with an analogue recorder BW-24 (Biochemical Science, Kanazawa, Japan) from electrolysis tissue blood flow meter RBA-2 (Biochemical Science). After incising the femur of the rats and exposing the sciatic nerve, we inserted the tip of a needle electrode BE-NS200–30 (Biochemical Science), and the hydrogen generated by electrolysis at that site was analyzed from the disappearance curves during constant time. The electrode was made by securing a Teflon-coated platinum-iridium wire (200 μmϕ). The insertion direction of the electrode was kept constant throughout measurement because slight variations in the obtained disappearance curves were noted that depended on the direction of insertion into the nerve, because the tip of the electrode was shaped like an injection needle.

Measurement of sciatic nerve blood flow was conducted at constant room temperature in the same room that was used for physiological investigations. Values of SNBF were calculated with the equation of Koshu et al. (15).

The rats were killed by dislocating their necks, and the sciatic nerve was excised. After measuring the wet weight of the sciatic nerve removed from the femur of the rats, we cryopreserved the nerve in a mortar with acetone and dry ice. The sciatic nerve was subsequently thawed, ground in a mortar containing 1 ml of 5% (wt/vol) $ZnSO_4$ with 10 μg/ml of D-(+)-arabitol and small amounts of sand, and mixed after the addition of 1 ml of 0.15 M $Ba(OH)_2$ aqueous solution. The supernatant was removed after centrifugation at 3000 rpm for 10 min at 4°C and was cryopreserved. Each sample was combined with 0.3 ml of pyridine, 0.2 ml of hexamethyldisilazane, and 0.1 ml of trimethylchlorosilane, and after being thoroughly mixed for 30 s, the samples were incubated for 1 h at 60–80°C. Then, 1 ml of chloroform and 2 ml of distilled water were added to the samples, and the mixture was stirred. After being centrifuged at 3000 rpm for 5 min at 4°C, the bottom layer was aliquoted and dried under N_2 gas flow. The dried samples were mixed after the addition of 0.1 ml of carbon disulfide, then analyzed by gas chromatography. For the peak area obtained by gas chromatography, the glucose, fructose, sorbitol, and *myo*-inositol content in the sciatic nerve was computed by comparison with the internal standard D-(+)-arabitol (16).

To measure serum lipids and serum glucose, we sampled blood from the tail vein (serum glucose values before dosing and on day 30 of dosing) and the abdominal aorta (day 60 of dosing). After being centrifuged at 3000 rpm for 10 min, only the serum was aliquoted and subjected to biochemical tests. Blood sampling was conducted while laboratory chow was freely available. For serum lipids, total cholesterol and triglyceride were measured by the enzymatic method (Determiner TC-S, TG-S, Kyowa Medex, Tokyo, Japan).

Serum insulin was measured by radioimmunoassay (Insulin Riabeads, Dainabot, Tokyo, Japan). Serum glucose content was measured with a spectrometer with the glucose C test (Wako Pure Chemicals, Osaka, Japan). During the test period, water intake and body weight were determined once a week.

Streptozocin (streptozocin for biochemical use, Wako Pure Chemicals) was used to induce diabetes. The test drug niceritrol (lot no. 71064/88) was provided by Sanwa Kagaku Kenkyusho (Nagoya, Japan). After the prescribed amount of niceritrol was weighed, it was suspended in 0.5% tragacanth gum solution (lot no. M7M1442, Nakarai Chemical, Kyoto, Japan) and administered.

After conducting analysis of variance, we performed multiple analyses with the Scheffe's test to compare the obtained results, and we also used the unpaired Student's *t* test. These data were expressed as means ± SE.

RESULTS

The changes in body weight and serum glucose levels for all groups of rats are shown in Table 1. Body weight and serum glucose levels in normal rats were similar in the CN group and the CC group. The rats with streptozocin-induced diabetes lost a significant amount of weight, but treatment with niceritrol (group DN) had no effect on weight loss or severity of hyperglycemia.

The results for serum lipids (total cholesterol, triglyceride) and serum insulin concentration after 8 wk of exper-

TABLE 1
Variations in body weight and serum glucose during the study

Animal group	Group code	−2 Wk Body weight (g)	Onset Body weight (g)	Onset Serum glucose (mM)	8 Wk Body weight (g)	8 Wk Serum glucose (mM)
Normal rats						
Control (n = 10)	CC	248.5 ± 2.1	356.1 ± 2.6	4.43 ± 0.04	556.0 ± 7.8	4.42 ± 0.08
Niceritrol treated (n = 8)	CN	243.8 ± 1.6	347.9 ± 3.2	4.51 ± 0.06	519.4 ± 12.8	4.60 ± 0.07
Diabetic rats						
Untreated (n = 8)	DC	248.0 ± 4.4	286.8 ± 5.0	20.0 ± 0.63	300.0 ± 11.4	17.8 ± 0.34
Niceritrol treated (n = 8)	DN	247.0 ± 3.7	281.0 ± 5.5	19.7 ± 0.56	251.9 ± 13.6	17.3 ± 0.62

Values are means ± SE. In all cases, $P < 0.001$ for differences between values for groups CC and DN, CC and DC, CN and DC, and CN and DN.

imentation are shown in Table 2. Total cholesterol values were lowest in group CN, showing a significant difference from those of groups DC and DN. A significant difference in total cholesterol levels was also observed between groups CC and DC, with group DC having the highest value of all four groups. Triglyceride values were highest in group DC, with a significant difference from groups CC, CN, and DN. Serum insulin values were lowest in group DN, with a significant difference from those of groups CC and CN. A significant difference in serum insulin levels was also observed between groups DC and CC and between groups DC and CN.

There were no differences in general status between groups CC and CN during the 8-wk experiment, including water intake. However, a significant increase in water intake was observed in the diabetic groups (DC and DN) compared with the groups of normal rats (CC and CN).

Administration of niceritrol for 8 wk significantly improved impaired MNCV in diabetic rats compared with untreated diabetic rats ($P < 0.001$; Table 3). However, there was no difference in MNCV between the two groups of normal rats. On the other hand, blood flow in the sciatic nerve was highest in group CN (16.8 ± 0.9 ml · min^{-1} · 100 g^{-1}) and lowest in group DC (6.4 ± 1.7 ml · min^{-1} · 100 g^{-1}), which showed a significant decrease in SNBF compared with groups CC, CN, and DN.

Thus, the data in Table 3 show the inhibitory effects of niceritrol on the development of impaired MNCV and SNBF in diabetic rats.

Glucose, sorbitol, and fructose concentrations in the sciatic nerve were markedly elevated in the diabetic rat groups DC and DN, with significant differences from the normal rat groups CC and CN (Table 4). There were no significant differences between groups CC and CN or between groups DC and DN. *myo*-Inositol content in the sciatic nerve was much lower in group DC; however, niceritrol significantly increased the concentration of *myo*-inositol in the sciatic nerves of the diabetic rats ($P < 0.01$). Although group CN had higher *myo*-inositol values than group CC, the differences were not significant.

DISCUSSION

In our study on rats with diabetes induced by streptozocin, the DN group showed no marked changes in serum total cholesterol, serum glucose, serum insulin concentration, and sugar (glucose, fructose, sorbitol) levels in nerve tissue. However, clear improvement was seen in MNCV and SNBF compared with values in the DC group after a marked decrease in serum triglyceride and a significant increase of nerve tissue *myo*-inositol content.

Niceritrol lowers serum lipid activity (17,18) and has peripheral vasodilatory effects (19). In our diabetic rat

TABLE 2
Total cholesterol, triglyceride, and insulin concentrations in serum of control and diabetic rats

Animal group	Group code	8 Wk Total cholesterol (mM)	8 Wk Triglyceride (mM)	8 Wk Insulin (μU/ml)
Normal rats				
Control (n = 10)	CC	2.30 ± 0.11	2.32 ± 0.28	20.5 ± 1.3
Niceritrol treated (n = 8)	CN	2.07 ± 0.10	2.34 ± 0.34	21.2 ± 1.6
Diabetic rats				
Untreated (n = 8)	DC	3.40 ± 0.21	4.56 ± 0.34	11.6 ± 0.9
Niceritrol treated (n = 8)	DN	3.00 ± 0.26	3.08 ± 0.26	9.1 ± 0.7

Values are means ± SE. Brackets indicate significantly different pairs of values.
a, $P < 0.001$; b, $P < 0.05$.

TABLE 3
Caudal motor nerve conduction velocity and sciatic nerve blood flow of control and diabetic rats

Animal group	Group code	8 Wk MNCV (m/sec)	8 Wk SNBF (ml · min^{-1} · 100 mg^{-1})
Normal rats			
Control (n = 10)	CC	31.0 ± 0.6	12.0 ± 1.2
Niceritrol treated (n = 8)	CN	31.8 ± 0.5	16.8 ± 0.9
Diabetic rats			
Untreated (n = 8)	DC	24.8 ± 0.9	6.4 ± 1.7
Niceritrol treated (n = 8)	DN	31.1 ± 0.7	15.3 ± 1.5

Values are means ± SE. MNCV, motor nerve conduction velocity; SNBF, sciatic nerve blood flow. Brackets indicate significantly different pairs of values.
a, $P < 0.001$; b, $P < 0.01$; c, $P < 0.05$; d, $P < 0.1$.

model, no clear effect of niceritrol on serum total cholesterol was noted, but serum triglyceride levels of group DN were significantly different from those of group DC. On the other hand, the SNBF of group DN improved as much as that of groups CN and CC. These results suggest that the action of this drug extends to the capillary level by changing the property of blood. Low et al. (20) investigated SNBF and endoneurial oxygen tension in rats with streptozocin-induced diabetes. According to their results, SNBF in the diabetic rats was 33% lower than in the control group. In response to this effect, partial oxygen pressure was reportedly also lower. Our results similarly show a marked decrease of 47% in the SNBF of the DC group compared with the CC group. Thus, we can infer that a decrease in partial oxygen pressure may be present in the nerve tissue of group DC, although this value was not measured in our study. Low et al. (20) also investigated the effect of oxygen supplementation on MNCV over 4 wk. The results showed that in the non–oxygen-supplemented group, a significant difference was observed in the MNCV value between streptozocin-treated rats and a normal control group, but no significant difference was seen in the oxygen-supplemented group. Thus, it appears that oxygen supply inhibits the delay in MNCV due to diabetes (20). This result suggests that oxygen supplementation accelerates glucose metabolism in nerve tissue for greater energy production. This assumption can also be inferred from the significantly lower values of free sugar in the nerve tissue of the oxygen-supplemented group compared with those of the non–oxygen-supplemented group. Dyck et al. (21) reported that patients with diabetic neuropathy have more occluded capillaries in the sural nerve than control subjects and patients without diabetic neuropathy, and that the percentage of occluded capillaries increases with the extent of impairment of neuropathy.

Because a decrease in erythrocyte deformability and an increase in erythrocyte cohesion with vascular endothelial cells has been reported in people with diabetes (22), blood properties and impairment of the vascular endothelium are considered causes of reduced blood flow in the nerve tissue of people with diabetes. Niceritrol reportedly decreases blood viscosity and inhibits accelerated platelet aggregation in diabetic subjects (23). Nakamura et al. (24) also observed an accelerating action of niceritrol on erythrocyte deformability in their clinical study. Moreover, Hamsten et al. (25) reported that the level of tissue plasminogen activator inhibitor in serum was positively and significantly correlated with levels of serum triglyceride. This finding suggests that high levels of serum triglyceride may inhibit fibrinolytic activity in the endothelial tissue of vessels.

TABLE 4
Free sugars in sciatic nerve of control and diabetic rats

Animal group	Group code	8 Wk Glucose	8 Wk Sorbitol	8 Wk Fructose	8 Wk *myo*-Inositol
Normal rats					
Control (n = 10)	CC	860.4 ± 248.3	41.1 ± 3.4	281.4 ± 52.1	674.1 ± 45.7
Niceritrol treated (n = 8)	CN	698.3 ± 231.2	35.1 ± 5.6	226.9 ± 46.0	819.0 ± 77.7
Diabetic rats					
Untreated (n = 8)	DC	2294.4 ± 267.9	262.1 ± 31.2	1712.6 ± 92.0	517.3 ± 50.1
Niceritrol treated (n = 8)	DN	3316.3 ± 373.8	299.4 ± 30.7	2096.6 ± 179.5	898.5 ± 67.3

Values are means ± SE of sugar concentration in nmol/100 mg dry tissue weight. Brackets indicate significantly different pairs of values.
a, $P < 0.001$; b, $P < 0.01$; c, $P < 0.05$; d, $P < 0.1$; e, $P < 0.05$ (Student's t test, CC vs. DC).

Our data showed reduced levels of serum triglyceride in group DN compared with group DC. We suggest that the beneficial effect of niceritrol on serum triglycerides led to improvement of blood flow disturbances caused by reduced fibrinolytic activity in nerve microvessels. Accordingly, we attribute the rise in SNBF to the results of improved blood fluidity. In any case, these improvements are thought to promote oxygen supply availability to nerve tissue, accelerate energy production in tissue, and inhibit delays in MNCV.

The inhibitory effect of niceritrol administration on delays in MNCV can also be explained by the marked rise of *myo*-inositol content in nerve tissue. Particularly in group DN, the mean *myo*-inositol value was higher than that of the groups CC and CN and was significantly different from that of group DC. Greene et al. (26) reported a decrease of *myo*-inositol content in the sciatic nerve with reduced MNCV in rats with streptozocin-induced diabetes. They simultaneously demonstrated that supplementing the diet of diabetic rats with *myo*-inositol prevents a decrease of *myo*-inositol content in the sciatic nerve and a delay in MNCV, despite high serum glucose levels and an accumulation of sorbitol and fructose in the sciatic nerve. The results for group DN in our study are similar to the results achieved with replenishment of dietary *myo*-inositol, suggesting that an increase in *myo*-inositol plays a large role in inhibiting delays in MNCV.

The details of the mechanism by which niceritrol increases *myo*-inositol content in nerve tissue, improves SNBF, and then inhibits a delay in MNCV are unknown. Moreover, another possible mechanism for the effects of niceritrol treatment exists. Although *myo*-inositol content in vascular tissues was not measured in our study, decreases there may also impair microvascular tissues and then induce a reduction of SNBF, resulting in the development of delayed MNCV. However, the alterations in microvascular *myo*-inositol metabolism brought about by the possible lipid-lowering effects of niceritrol might be responsible for the changes in nerve blood flow, leading to the improvement of delayed MNCV.

The improvement in sciatic nerve blood flow and the increased *myo*-inositol content in nerve tissue caused by niceritrol administration seen in our study suggest that both vascular and metabolic factors play an important role in diabetic neuropathy. We hope that further research will be conducted on this subject.

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